

1 ***Supplementary information***

2 Substrate-driven convergence of the microbial community in lignocellulose-amended
3 enrichments of gut microflora from the Canadian beaver (*Castor canadensis*) and North
4 American moose (*Alces americanus*)

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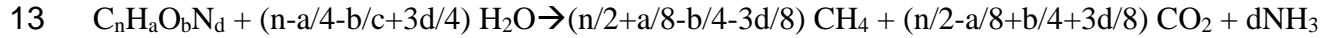
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10 **1. Supplemental methods**

11 Calculation of stoichiometric maximum biogas volume per mg COD carbohydrates:

12 According to Buswell's equation (Symons and Buswell, 1933),



14 Using glucose as an example for carbohydrate, we have $C_6H_{12}O_6 \rightarrow 3 CH_4 + 3 CO_2$.

15 Assuming ideal conditions and no dissolution of CO_2 in the medium, 1 mole of glucose yields 6
16 mole of gases. Using the ideal gas law ($PV = nRT$ or $V = nRT/P$), 1 mole of glucose yields 6
17 $mol \times 298 K \times 0.082057 L atm K^{-1} mol^{-1} \div 1 atm = 146.7 L$ biogas, or 1 mg of glucose yields
18 0.8142 ml biogas. Consider the oxidation of glucose: $C_6H_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O$, the
19 chemical oxygen demand (COD) of glucose = $6 \times 32 g/mol O_2 \div 180.1559 g/mol$ glucose =
20 1.066 g O_2/g glucose. Therefore, the stoichiometric maximum biogas yield is 0.8142 ml
21 biogas/mg glucose $\div 1.066 g O_2/g$ glucose = 0.764 ml biogas/mg COD.

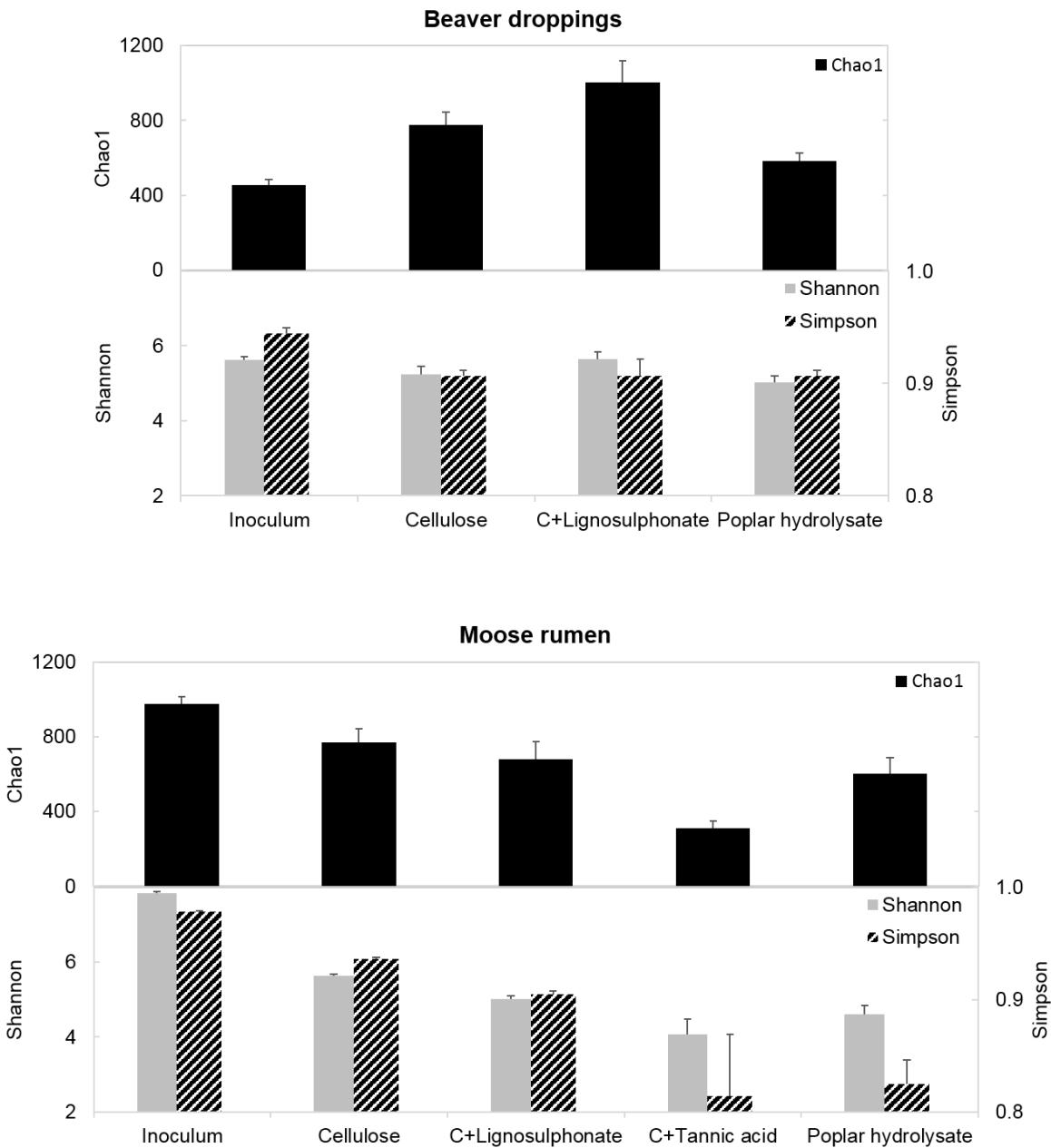
22 Consider sodium lignosulphonate ($C_{20}H_{24}Na_2O_{10}S_2$) (National Center for Biotechnology
23 Information, 2016b), 1 mole of this substrate yields 489.1 L biogas containing 10.5 mole of CH_4

24 and 9.5 mole of CO₂. Based on the measured COD of sodium lignosulphonate (Table S1), the
25 stoichiometric maximum biogas yield is 0.58 ml biogas/mg COD. Similarly, 1 mole of tannic
26 acid (C₇₆H₅₂O₄₆) (National Center for Biotechnology Information, 2016a) generates 1858.4 L
27 biogas containing 33 moles of CH₄ and 43 mole of CO₂ based on stoichiometry, and the
28 calculated maximum biogas yield is 0.90 ml biogas/mg COD using the measured COD of tannic
29 acid (Table S2). In proportion of the substrates added at each enrichment phase, the
30 stoichiometric maximum biogas yields (ranged from 0.69 to 0.82 ml biogas/mg COD substrate)
31 were derived (Table S2). Actual yield may differ owing to factors such as limited
32 biodegradability of compounds and solubility of CO₂ in liquid.

- 33 **2. Supplementary Data**
- 34 **Supplementary Data 1** Summarized relative abundances of microbial lineages in beaver
35 dropping, moose rumen and their enrichment microcosms.
- 36 **Supplementary Data 2** Taxonomic assignment of the OTUs defined at 97% nucleotide identity.

37 3. Supplementary Figure and Tables

38 3.1 Supplementary Figure



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40 **Figure S1** Diversity indices of inocula and corresponding enrichments amended with various
41 lignocellulosic substrates. Error bars indicate standard deviation; n=3.

42 **3.2 Supplementary Tables**

43 **Table S1** Measured chemical oxygen demand (COD) of enrichment substrates (\pm standard
44 deviation).

Enrichment substrate	COD content (g COD/g substrate)
Cellulose	1.22 \pm 0.12
Sodium lignosulphonate	1.54 \pm 0.11
Tannic acid	1.22 \pm 0.00
Poplar hydrolysate	0.93 \pm 0.30

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46 **Table S2** Lignocellulosic amendments for enrichment microcosms and stoichiometric maximum biogas yields.

Enrichment	Cellulose (C)		Lignosulphonate (L), Tannic acid (T), or Poplar hydrolysate (PH)		Total COD (mgCOD)	Estimated stoichiometric maximum biogas yield (ml biogas/mg COD substrate)
	mg per bottle	mgCOD per bottle	mg per bottle	mgCOD per bottle		
Phase 1						
C	28.4	33.7	-	-	33.7	0.76
CL	22.8	27	5.3	8.1	35.1	0.72
CT	22.8	27	17.7	22	49	0.82
PH	-	-	28.4	26.6	26.6	-
Phase 2-3						
C	142.2	168.5	-	-	168.5	0.76
CL	113.8	134.9	26.3	40.5	175.4	0.72
CT	136.5	161.8	17.7	22	183.8	0.78
PH	-	-	142.2	132.8	132.8	-
Phase 4						
C	113.8	134.9	-	-	134.9	0.76
CL	113.8	134.9	26.3	40.5	175.4	0.72
CT	113.8	134.9	88.5	109.9	244.8	0.82
PH	-	-	136.5	127.5	127.5	-
Phase 5-10						
C	125	152.5	-	-	134.9	0.76
CL	125	152.5	90	109.8	262.3	0.69
CT	125	152.5	26.3	40.5	193	0.79
PH	-	-	136	126.5	126.5	-

48 **Table S3** Dates and duration of microcosm enrichment phases 1 to 4, 7, and 9.

	Inocula Substrates	Beaver dropping				Moose rumen			
		C	CL	CT	PH	C	CL	CT	PH
Phase 1	Start date ¹	10-02-12	10-02-12	10-02-12	10-02-12	09-10-13	09-10-13	09-10-13	09-10-13
	Duration (d)	124	124	124	124	201	201	201	201
Phase 2	Start date	10-07-23	10-07-23	10-07-23	10-07-23	10-08-12	10-08-12	10-08-12	10-08-12
	Duration (d)	55	55	55	55	62	62	62	62
Phase 3	Start date	10-09-30	10-09-30	10-09-30	10-09-30	10-11-17	10-11-17	10-11-17	10-11-17
	Duration (d)	90	90	90	90	58	58	58	58
Phase 4	Start date	11-01-06	11-01-06	11-01-06	11-01-06	11-01-15	11-01-15	11-01-15	11-01-15
	Duration (d)	30	30	30	30	30	30	30	30
Phase 7	Start date	11-12-05	11-12-05	11-12-05	11-12-05	11-12-05	11-12-05	11-12-05	11-12-05
	Duration (d)	259	176	259	259	259	176	259	259
Phase 9	Start date	12-11-06	12-11-12	12-11-01	12-10-20	12-11-06	12-11-08	12-11-01	12-10-20
	Duration (d)	78	71	82	94	78	75	82	94

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¹Dates are displayed as YY-MM-DD.

50 **Table S4** Multiplex barcodes, DNA concentration, and numbers of reads for amplicon samples
 51 prepared from beaver dropping, moose rumen and their enrichment microcosms.

Enrichment condition	Multiplex barcode	Amplicon DNA concentration (µg/ml)	Number of reads
Beaver dropping			
Inoculum	TAGTGTAGAT	31.7	4504
	TCGCACTAGT	38.2	5116
	TCTATACTAT	43.4	5213
Cellulose	ACATACGCGT	138	7471
	ACGCGAGTAT	116.3	7828
	ACTACTATGT	168.9	8312
Cellulose+Lignosulphonate	TACGAGTATG	158.9	9475
	TAGAGACGAG	133.1	8191
	TCGTCGCTCG	90.3	5921
Cellulose+Tannic acid	ATAGAGTACT	29.1	-
	CACGCTACGT	30.5	-
Poplar hydrolysate	AGTACGCTAT	99.1	8699
	ATAGAGTACT	116.4	9232
	CACGCTACGT	51.2	4441
Moose rumen			
Inoculum	CGACGTGACT	34.2	3198
	TACACGTGAT	25.8	3444
	TACGCTGTCT	20.1	5339
Cellulose	TGTACTACTC	154.9	8051
	ACGACTACAG	133.8	6972
	CGTAGACTAG	163.6	8523
Cellulose+Lignosulphonate	TGATACGTCT	154.3	8694
	CGAGAGATAC	116.9	6378
	TCTACGTAGC	129.4	7100
Cellulose+Tannic acid	AGCGTCGTCT	23.6	9144
	AGTACGCTAT	65.2	9128
Poplar hydrolysate	CAGTAGACGT	85.2	8522
	TACACGTGAT	68.2	5739
	TACGCTGTCT	100.5	8673

53 **Table S5** Statistics of 16S rRNA pyrotag sequencing runs.

Sample	Total number of reads	Number of OTUs	Number of OTUs with abundance $\geq 0.5\%$ (sum of represented abundances)
Beaver droppings			
Inoculum	14564	415	22 (72.9%)
Cellulose	23268	685	21 (77.6%)
Cellulose+Lignosulphonate	23024	840	23 (70.2%)
Cellulose+Tannic acid ¹	12	-	-
Poplar hydrolysate	22077	581	21 (77.9%)
Moose rumen			
Inoculum	11291	967	29 (42.2%)
Cellulose	23091	734	22 (73.3%)
Cellulose+Lignosulphonate	21869	644	18 (76.4%)
Cellulose+Tannic acid	18003	346	17 (80.5%)
Poplar hydrolysate	22614	588	18 (76.0%)

54 ¹ Dataset for beaver droppings enriched on cellulose plus tannic acid was removed from downstream analysis due to low number of
 55 reads (less than 15).

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57 **Reference**

- 58 National Center for Biotechnology Information (2016a). *PubChem Compound Database*;
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- 61 National Center for Biotechnology Information (2016b). *PubChem Compound Database*;
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63 <https://pubchem.ncbi.nlm.nih.gov/compound/25113562> [Accessed 24 April 2016].
- 64 Symons, G.E., and Buswell, A.M. (1933). The methane fermentation of carbohydrates. *J Am
65 Chem Soc* 55:2028-2036. doi: Doi 10.1021/Ja01332a039.
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